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13. Abstract (Maximum 200 Words) *(abstract should contain no proprietary or confidential information)*

The long term **objective** of this work is to elucidate metabolic pathways which can be used to reduce the need for radical surgery in patients at high risk for prostate cancer or with early stage disease. The **hypothesis** to be tested is that alterations to lipoxygenase (LOX) and cyclooxygenase (COX) activity in early prostate cancer represent distinct druggable pathways which can be treated in conjunction with the PPAR γ signaling pathway to slow or prevent the development and progression of prostate cancer. In this final report we summarize the work performed over the life of the grant with details limited to the no cost extension period. We demonstrated the loss of PPAR γ in a prostatic conditional knockout model. We showed that the combination of PPAR γ loss with other common genetic insults can cause progression to a PIN phenotype, and that PPAR γ loss in human epithelial cells results in phenotypic changes including both PIN and urothelial differentiation. We have demonstrated that changes in 15-lipoxygenase-1 and -2 expression can elicit changes in prostatic morphology, specifically premalignant lesions, as initially proposed. These findings validate the potential for chemopreventive uses for PPAR γ agonists. During the life of the grant unexpected side effects of the TZD PPAR γ agonists resulted in the withdrawal of these drugs from the market. We are investigating this as well as clinical links between TZD use and prostatic disease under funding from NIH. Results from the studies under this DOD-PCRPP grant suggest the need for a new class of drugs to activate PPAR γ for use in prostate cancer chemoprevention.

14. Subject Terms (keywords previously assigned to proposal abstract or terms which apply to this award)

PPAR γ , lipoxygenases, cyclooxygenases, oxidative stress

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Final Report

PCRP Idea Development Award

W81XWH-07-1-0479

Modulation of PPAR-Gamma Signaling in Prostatic Carcinogenesis

P.I. Simon W. Hayward, PhD

Introduction

This project set out to examine the relationship between PPAR γ and carcinogenesis. PPAR γ sits at a critical juncture in cellular differentiation and metabolism being involved in both differentiation and in the regulation of stress responses mediated through the cyclooxygenase (COX) and lipoxygenase (LOX) pathways of fatty acid metabolism.

The basis for this work was the observation that in human prostate cancer there is an early loss of enzymes responsible for the production of the putative endogenous ligands for PPAR γ , presumed to result in a decrease in receptor function. We have found that loss of PPAR γ function can result in the generation of premalignant prostatic lesions in mice (Jiang et al 2010). We have also shown that there is an associated upregulation of COX pathways which would generate increases in prostaglandin production and oxidative stress, which could underlie such pathology. We set out to examine interactions between the PPAR γ , COX and LOX pathways and their role in carcinogenesis. To pursue the work in human cells we have developed two new human prostatic epithelial cell lines (NHPrE1 and BHPrE1) to serve as a basis for *in vivo* studies of human prostate. We used predominantly tissue recombination models involving human prostatic epithelial cells. The use of human cells is important in that there are significant differences between the fatty acid metabolic pathways between humans and mice. However we have also generated mouse epithelial cell lines from the transgenic animals and as a result have been able to use their accelerated aging and metabolism as compared to human cells to illustrate malignant transformation in a recombination model. These data provide a strong basis for future studies

Body

Status of progress in relation to the original SOW

Task 1. Examine the in vivo consequences of suppression of PPAR γ signaling in human prostatic epithelium.

This task is completed, as described in the annual reports.

Task 2. Examine the in vitro and in vivo consequences of overexpression of cyclooxygenase -1 or -2 or 15-lipoxygenase-1 in human prostatic epithelium.

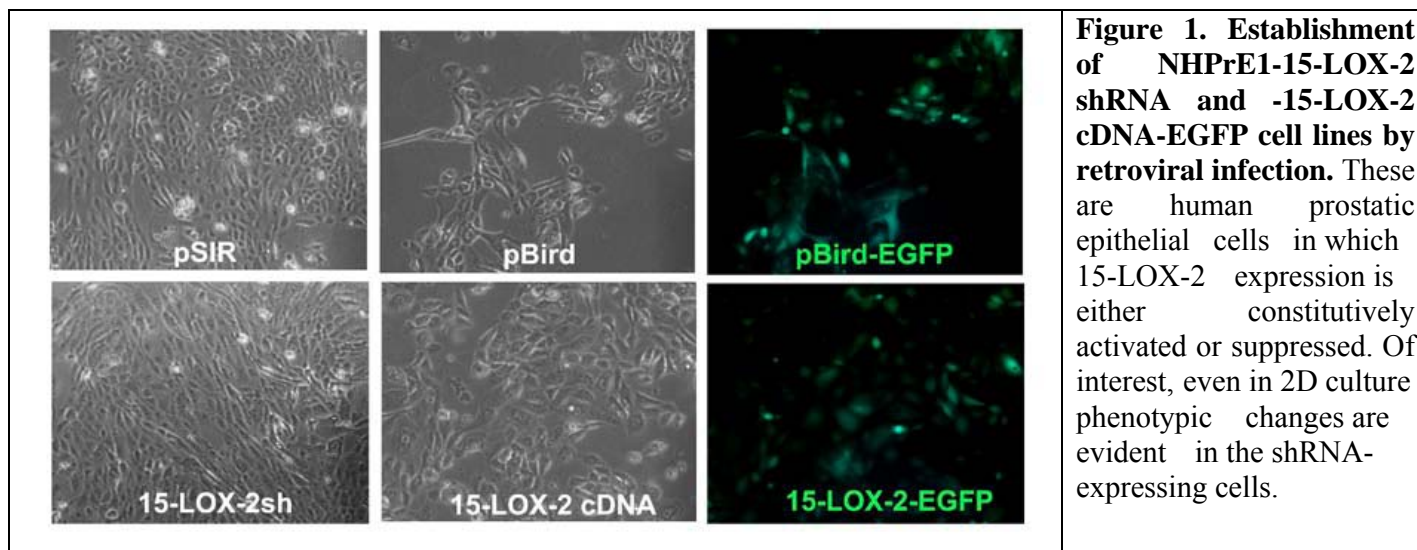
These studies were modified slightly, as noted in the second annual report by moving to our new human cell lines. The data analysis is completed and will form a part of a manuscript which is in preparation which also incorporate studies relating to task 3.

Task 3. Examine protective effects of PPAR γ agonists and/or COX/LOX inhibitors against the neogenesis of PIN or progression of prostate cancer.

These studies are now completed and a final manuscript including these data and data from task 2 is in preparation.

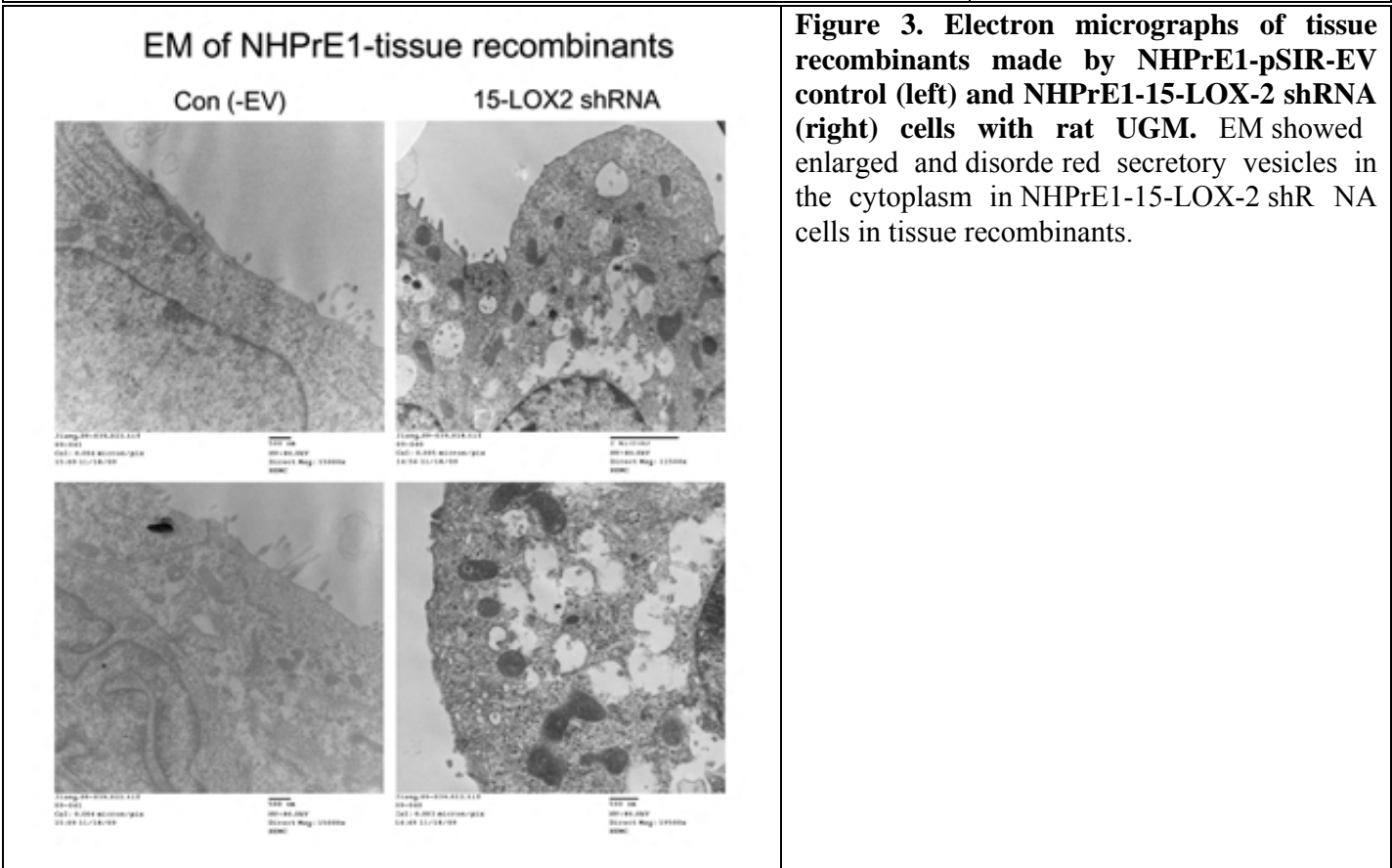
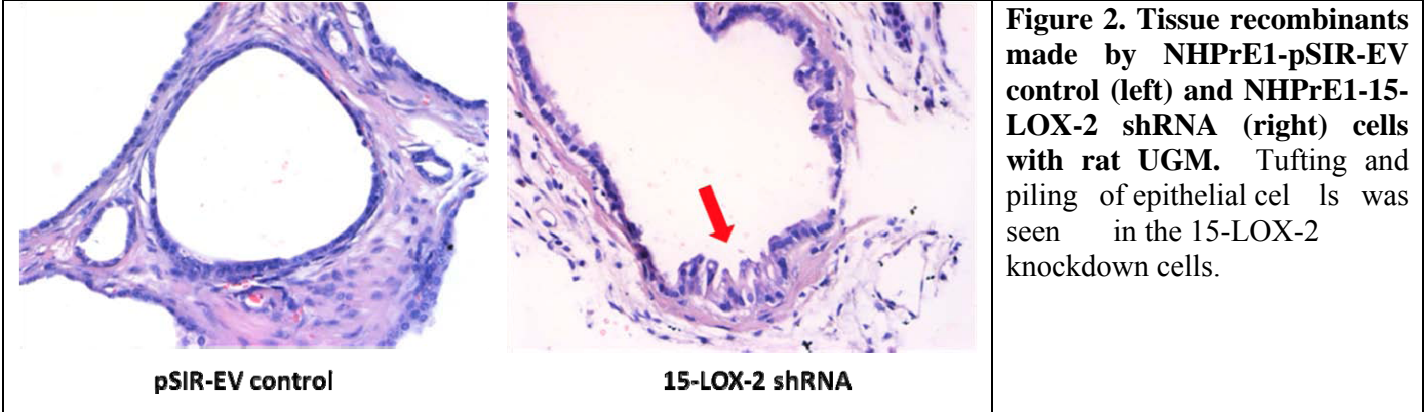
Summary of Activity

The scientific activity in this grant was mostly described in the previous annual reports and publications emerging. Here we will report only on unpublished observations made in the no cost extension period.



The cell lines required to generate the final sets of tissue recombinants were generated with two examples shown in figure 1. These were based upon the previously-described NHPeE1 line.

Tissue recombinants were generated to test the effects of manipulation of 15-LOX-2 in prostate tissue *in vivo*. These studies showed the development of PIN-like lesions in 15-LOX-2 knockdown cells (figure 2) consistent with previous studies of PPAR γ knockout or suppression. This supports the idea that loss of PPAR γ is functionally similar to loss of its putative ligand as we originally proposed. Changes at the cytologic level are shown in figure 3.



Similar experiments were performed to overexpress the enzyme 15-LOX-1 in the same cells (figure 4). As predicted this resulted in changes in consistent with PIN in the epithelial cells. The balance between 15-LOX-1 and 15-LOX-2 activity is postulated to play a key role in the activation of PPAR γ and thus

epithelial cell differentiation. These observations are consistent with that initial premise.

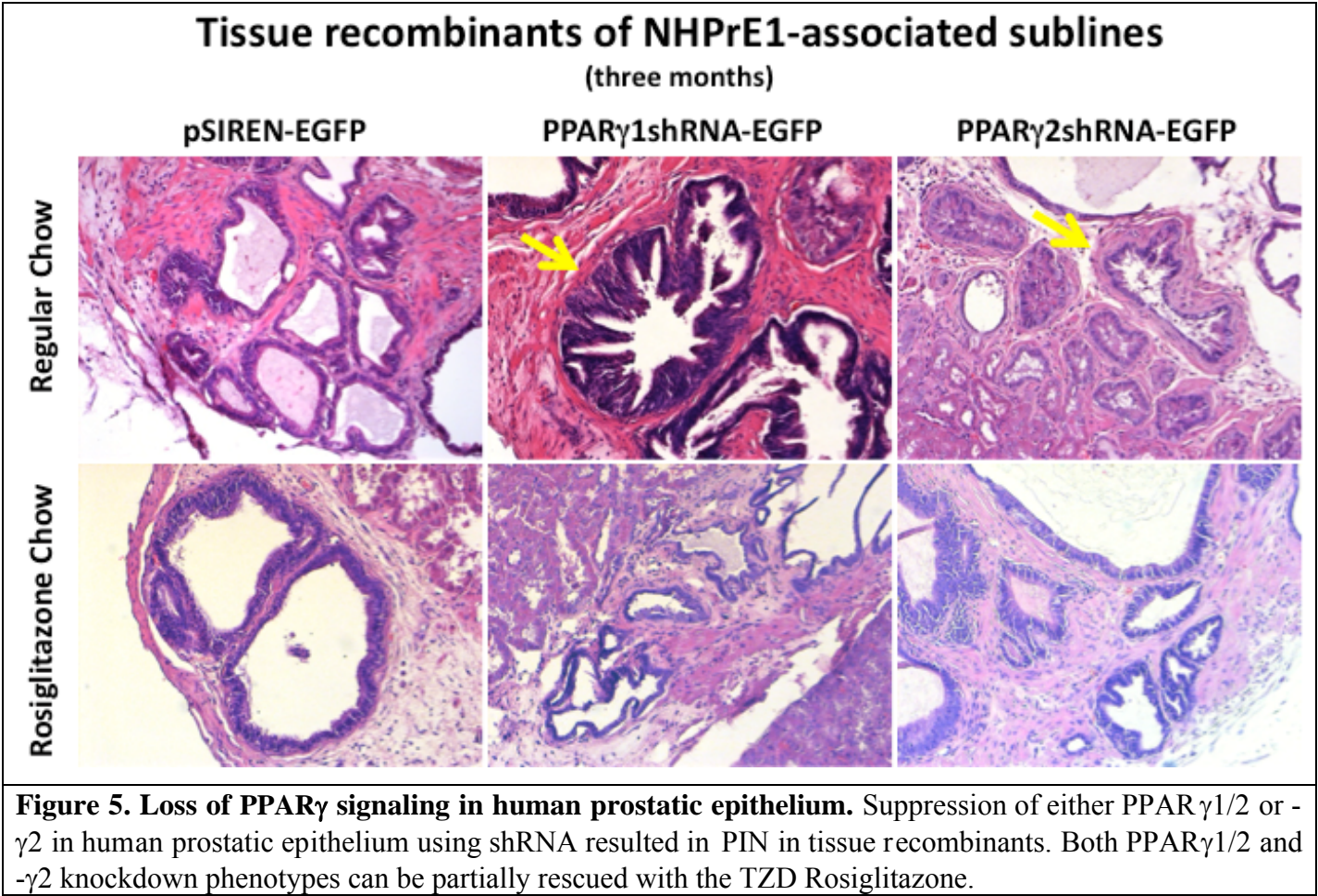
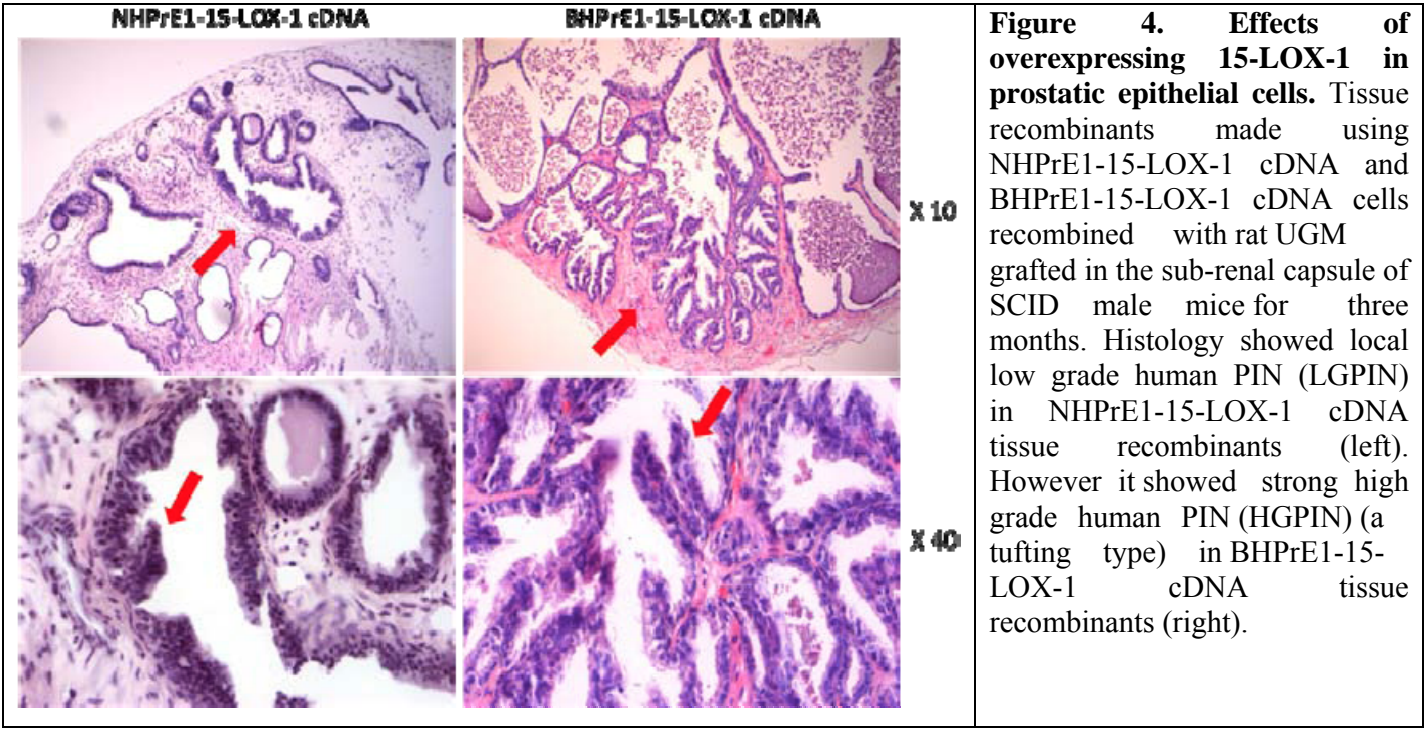


Figure 5. Loss of PPAR γ signaling in human prostatic epithelium. Suppression of either PPAR γ 1/2 or - γ 2 in human prostatic epithelium using shRNA resulted in PIN in tissue recombinants. Both PPAR γ 1/2 and - γ 2 knockdown phenotypes can be partially rescued with the TZD Rosiglitazone.

To examine the role of PPAR γ signaling in prostatic carcinogenesis we recapitulated the transgenic mouse model using human epithelial cells in which PPAR γ expression was knocked down using shRNA to target either total PPAR γ or PPAR γ 2 specifically. In both cases we were able to show the formation of PIN-like lesions in tissue recombination models which could be rescued using the TZD Rosiglitazone as a chow supplement (figure 5). This is consistent with the lipoxxygenase data shown above and reinforces the idea that PPAR γ signaling can play a protective role by maintaining prostatic differentiation.

Key Research Accomplishments (over entire project life)

These accomplishments represent a summation of those described in the three annual reports.

- Fully characterized and described mice with conditional knockout of PPAR γ in the prostate. This description was published in *Cell Death and Differentiation* in 2010 (reference cited in reportable outcomes section).
- Generated and described two new human prostate epithelial cell lines (NHPrE1 And BHPPrE1). These represent a powerful tool that can be used to investigate many aspects of both benign and malignant prostatic disease. This is a huge improvement on the previously existing lines and fills a critical need for research by retaining the ability to express all of the key markers of prostate epithelial function (notably androgen receptors and PSA). These cells have already been freely distributed to many laboratories following requests. Description published in *Stem Cells* in 2010 (reference cited in reportable outcomes section).
- Established that loss of PPAR γ leads to autophagy in the conditional knockout mouse. Further that such autophagic changes are associated with malignant progression.
- Findings that loss of PPAR γ result in both autophagy and inflammation were confirmed in tissue recombination models using PPAR γ -KO epithelial cell lines. As these lines age they give rise to cancer in tissue recombination models, suggesting that accumulation of insults with time is a potentially transforming event, and further supporting our contention that loss of this pathway can be critical in prostatic carcinogenesis and that activation of PPAR γ might be a worthwhile chemopreventive approach.
- Observations in the human model demonstrated the key role that PPAR γ can play in contributing to epithelial cell differentiation. This work suggests a key role for the pathway in cellular commitment to specific lineages. This unexpected result clearly has significance for a basic understanding of cellular biology, but is of less immediate impact for prostate cancer research.
- Generated knockdown of PPAR γ -1/-2 by siRNA in human prostatic epithelial cells. Demonstrated that in tissue recombination models these undergo similar profiles of phenotypic changes to those seen in mouse prostate in which expression of this gene is suppressed, notably with the consistent expression of a PIN phenotype.
- Generated cells in which both PPAR γ -1/-2 and PTEN expression were suppressed.
- Generated human prostatic epithelial cells overexpressing 15-LOX-1 and suppressing 15-LOX-2, and their functional opposites. In tissue recombination experiments data shows the key role played by 15-LOX-2 in maintaining prostatic differentiation and the ability of high levels of 15-LOX-1 to disrupt

this function.

Reportable Outcomes (over project life)

The following publications have been supported in whole or in part from this research grant:

Jiang, M., Strand, D.W., Fernandez, S., He, Y., Yi, Y., Birbach, A., Qiu, Q., Schmid, J., Tang, D.G. and Hayward, S.W. [2010] Functional Remodeling of Benign Human Prostatic Tissues *in vivo* by Spontaneously Immortalized Progenitor and Intermediate Cells. *Stem Cells* 28, 344-356

Jiang, M., Fernandez, S., Jerome, W. G., He, Y., Yu, X., Cai, H., Boone, B., Yi, Y., Magnuson, M. A., Roy-Burman, P., Matusik, R. J., Shappell, S. B. and Hayward, S. W. [2010] Disruption of PPAR γ signaling results in mouse prostatic intraepithelial neoplasia involving active autophagy. *Cell Death and Differentiation* 17, 469-481

Jiang, M., Jerome, W.G. and Hayward, S.W. [2010] Autophagy in nuclear receptor PPAR γ -deficient mouse prostatic carcinogenesis. *Autophagy* 6, 175-176

Strand, D.W., Franco, O.E., Basanta, D., Anderson, A.R.A., Hayward, S.W. [2010] Perspectives on Tissue Interactions in Development and Disease. *Current Molecular Medicine* 10, 95-112

www.UroToday.com. Beyond the Abstract - Functional remodeling of benign human prostatic tissues *in vivo* by spontaneously immortalized progenitor and intermediate cells by Ming Jiang, MD, PhD., Monday, 08 March 2010.

http://www.urotoday.com/3345/browse_categories/beyond_the_abstract/beyond_the_abstract_functional_remolding_of_benign_human_prostatic_tissues_in_vivo_by_spontaneously_immortalized_progenitor_and_intermediate_cells_by_ming_jiang_md_phd03082010.html

Conclusions

This proposal was very productive in terms of the tools and publications generated. Perhaps the most important outcome for the field of prostate cancer biology is the development of the new epithelial cell lines. This represented tool development for this project but clearly has greater implications for the research community, giving us the ability to make targeted mutations in human prostate epithelium and to investigate the consequences of these without the confounding background of viral oncogenes or the excessive anaplastic nature of the established cancer lines.

We were able to demonstrate the key role played by PPAR γ in the differentiation of the prostate and to show that loss of this signaling pathway resulted in autophagy in the mouse prostate, subsequently confirmed in human cells. This provides a mechanism for the development of pre-malignant lesions presumably due to the acquisition of genetic or epigenetic hits resulting from the induction of inflammation and oxidative stress caused by PPAR γ suppression. The results supported our contention that activation of PPAR γ may be protective and represent a potential target for chemoprevention. Some of this work is now being followed up under funding from the NIDDK which is examining the links between PPAR γ action, lipid metabolism and inflammation. While the primary focus of that work is benign prostatic hyperplasia and LUTS there are obvious spinoffs into the field of prostate cancer research.

As discussed in the third annual report, one of the predicates when we wrote the initial proposal was that, given positive findings, the work would be quickly translatable since there were a number of glitazone drugs on the market specifically designed to agonize the PPAR γ pathway. Unfortunately in the intervening period most of these have been pulled from the market due to off target toxicity, including notably bladder cancer. There is a widespread enthusiasm among clinicians in the field of diabetes where these drugs have been most widely used that there is a pressing need for new drugs targeting PPAR γ . Epidemiologic studies at Vanderbilt are also examining the effects of both glitazone and metformin use in diabetic patients on subsequent diagnoses of BPH/LUTS or of prostate cancer. The outcomes of these studies may also contribute to changing the thought process on whether such approaches can be used to target multiple common co-morbidities of diabetes, including BPH/LUTS and possibly also answer the question of whether these compounds are chemopreventive for prostate cancer. Such a finding should spur research in the development of new drugs to directly or indirectly target the pathway.

The final sets of experiments have now been harvested and the data are under going analysis. Given that the funding under this mechanism is now exhausted we will complete the write up of these data for publication independently of this grant, however the grant will be credited in the acknowledgments section.

Data related to this project were reported at the recent IMPACT meeting in Florida.